ON HAEMATOZOA OCCURRING IN WILD ANIMALS IN AFRICA.

1. *PIROPLASMA ROSSI* N.SP. AND *HAEMOGREGARINA CANIS ADUSTI* N.SP. FOUND IN THE JACKAL.

2. *SPIROCHAETA BOVIS CAFFRIS* N.SP. FOUND IN THE BUFFALO.

BY GEORGE H. F. NUTTALL, F.R.S.

Plates X and XI.

OWING to the courtesy of my friend, Mr W. F. Cooper, B.A., who prepared blood-films and organ smears from various wild animals shot by him in British East Africa in August, 1909, I am in a position to record three new parasites from the Jackal and Buffalo. Mr Cooper prepared the films as soon as possible after the animals were shot, using either absolute alcohol or heat for fixation. On arrival in Cambridge, the films were stained in the usual manner by Giemsa.

The Jackal (*Canis adustus*) was shot to the S.E. and near to Fort Hill, about four miles on the road to Punda Milia. The Buffalo (*Bos caffer typicus*) was shot about five miles to the north of Punda Milia. I shall first describe the two protozoal parasites encountered in the Jackal.

The Parasites of the Jackal.

The parasites were encountered in the course of a very careful and systematic examination of eight coverglass smear preparations made from the spleen (4) and liver (4) of the jackal. Altogether, the smeared coverglasses represented an area of 12 square centimetres and it took many days to thoroughly examine them with the aid of a mechanical stage. My laboratory assistant, Mr B. G. Clarke, aided me considerably in the search for parasites. In all, we discovered 98 parasites, of which eight were haemogregarines.

Piroplasma rossi n.sp.

Plate X.

It is with hesitation that I place this parasite in the genus *Piroplasma*, and I only do so provisionally for reasons about to be described. On the other hand, it appears safe to refer the protozoon to the Family *Piroplasmidae* of França. Were it not for the fact that I have recently discovered somewhat similar parasites in the internal organs of a dog 235 days after it had been inoculated with *Piroplasma canis*, the dog having recovered in consequence of trypanblue treatment (see *Parasitology*, Vol. II. p. 180), I should unhesitatingly have referred the parasite of the jackal to a new genus. Only two parasites were encountered in the dog, and it will be necessary to examine other animals before any conclusions can be reached regarding the resemblance existing between the parasites. The parasites of the jackal differ very markedly from *P. canis* as observed in the peripheral blood during the normal course of piroplasmosis. Thus, in the jackal parasite there is an absence of the typical pairs of pyriforms and the characteristic dividing ("budding") forms are lacking; the nucleus is, moreover, very much larger and differs from that of *P. canis*, as elsewhere described by Dr Graham-Smith and myself. When we come to examine smears from the organs of dogs, long after the animals have "recovered," with the same care that we have devoted to the examination of the smears from the jackal, we shall be in a better position to express an opinion on the subject. It is conceivable that the parasites of the jackal represent a special form of *Piroplasma* occurring in animals which suffer from a chronic infection or which have recovered from acute piroplasmosis. To my knowledge parasites have not hitherto been described from animals after "recovery" from piroplasmosis. We know that they are present in peripheral blood because the blood of "salted" animals is infective for months or years although failure has attended the efforts made to detect the parasites by microscopic examinations. Assuming, on the other hand, that the parasite of the jackal is a *Piroplasma* of an hitherto undescribed type and peculiar to recovered animals, it may well be referred to a new species in view of the host in which it is found, for, as we have recorded elsewhere, the African *P. canis* appears to be peculiarly specific since infection experiments made with it upon jackals and foxes (*Parasitology*, Vol. II. p. 211) have given negative results. Should future investigation prove that the parasite of the jackal differs from the types of *P. canis* encountered in "salted" dogs, I would propose to refer it to a new genus *Rossiella*[1] (*Rossia* is preoccupied) in view of its specific name.

The following description is based upon the examination of 90 parasites encountered after a complete search had been made of the stained preparations at my disposal. The spleen contained 84 parasites, the liver smears only six parasites.

P. rossi occurs as a large intracorpuscular parasite of rounded, ovoid, sub-pyriform or irregular shape, possessing a single large nucleus, and occurring singly, in pairs or in fours, within the infected corpuscles. The parasite produces no pigment and appears to multiply by simple division into two parasites, which may in turn subdivide. Simple division of the nucleus precedes the separation into daughter cells. Judging from the stained preparations there is evidence that the parasite is capable of amoeboid movement at various stages of its development. The parasite causes no apparent changes in the corpuscles which it inhabits.

Measurements. The smallest uninucleate parasites measured $1{\cdot}5\,\mu$, the largest $3{\cdot}3 \times 5{\cdot}3\,\mu$. Since the large parasites subdivide to form two or four smaller parasites intermediate sizes are encountered with nuclei of corresponding size. Both the infected and uninfected red blood corpuscles varied in size, from 6 to $9\,\mu$ in diameter.

General structure. The general form of the parasite varies considerably. It may be spherical and sharply defined (Pl. X, Figs. 1, 5, 16) or ovoid (Figs. 4, 10, 25) to sub-pyriform (Figs. 7, 13, 14), or it may be irregular in outline owing to the protrusion of amoeboid processes which are lobose (Figs. 3, 15) or more or less pointed (Figs. 8, 12, 19, 26). The *protoplasm* takes a clear blue colour, when stained by Giemsa, with a tendency for the staining to be more intense at the periphery, as in *Piroplasma*, but there is less evidence of vacuolar structure; in fact the protoplasm may at times assume a uniformly blue tint. In all the parasites a clear unstained halo surrounds the chromatin mass. At times clear vacuolar areas are seen in the blue staining protoplasm (Figs. 1, 3, 15), at other times fairly definite achromatic lines traverse the parasite (Figs. 16, 17). The *nucleus* is striking because of its relatively large size. It is situated centrally or peripherally; in the latter case it is in most instances bounded on the outside by a band of blue-staining protoplasm. The nucleus is usually of rounded or oval form with a sharply defined contour. In some nuclei, fine chromatin particles appeared to be aggregated at the periphery or to be scattered through the pale pink-staining substance of the chromatic nucleus. When nuclear division occurs (see below under Mode of Multiplication) the chromatin appears to be more diffused and the nucleus is of irregular form. In one case the extrusion of what may have been a dividing nucleus was observed (Fig. 15). In this case the chromatin is peripherally distributed as though surrounding a vacuole, but the appearance may be unnatural, especially as the blue-staining parasite itself appears "blistered," as is occasionally seen in *Piroplasma* in imperfectly stained films.

---

[1] In honour of Professor Ronald Ross, C.B., F.R.S.

Mode of Multiplication. The manner in which the parasite undergoes division within the corpuscle appears to be as follows: when a single parasite (Pl. X, Figs. 1—4) has attained a certain size, its nucleus divides (Figs. 5—6) and this is followed by division of the parasite into two (Figs. 7—12). The newly-formed pair of parasites, if sufficiently mature, may repeat the process (Figs. 15—19), thus giving rise to four parasites within a corpuscle (Figs. 20—22). Nuclear division is simple and clearly precedes the division of the protoplasm both in single parasites (Figs. 5—6) and in pairs of intracorpuscular parasites (Figs. 15—19). In parasites about to divide the chromatin of the nucleus appears to be diffuse (Fig. 4) or drawn out in an irregular thread-like manner (Figs. 13, 14) as if the nucleus were in a state of flux. The chromatin becomes aggregated at opposite ends of the parasite and the connecting strand ruptures (Fig. 15), after which the newly-formed nuclei become rounded (Figs. 15—19).

In view of the small number of parasitized corpuscles which could be discovered in the film it is impossible to say if more than four parasites may be formed in the corpuscle. Judging, however, from the absence of smaller forms than those figures (Figs. 18, 23, 29) it is reasonable to assume that not more than four parasites are formed within a corpuscle.

Enumeration of the Types of Parasites encountered.

Intracorpuscular parasites. The complete examination of the smears from the jackal only revealed 90 parasites. Sketches and notes were made of all the parasites encountered. In ordering the data thus collected it was found that the majority of the infected corpuscles (81 °/₀) contained one, two or four discrete parasites, whereas the remaining corpuscles contained parasites in various stages of development, as shown (bracketed "D") in the subjoined table:

	No. of r.b.c.	which contained parasites as follows	As figured in Plate X
81 %	33	2 parasites each with a single nucleus.	Figs. 7—12.
	29	1 parasite with a single nucleus.	Figs. 1—4, 23, 24.
	11	4 parasites each with a single nucleus.	Figs. 20—22.
9 % (D)	6	1 parasite with two nuclei.	Figs. 5—6.
	3	2 parasites each with two nuclei.	Figs. 15—17.
	3	2 parasites each with nuclei showing processes.	Figs. 13—14.
	3	3 parasites: two containing single nuclei and one containing two nuclei.	Figs. 18—19.
	2	1 parasite with nucleus showing processes.	As in Fig. 14.

Free parasites. With but one exception all of the parasites which were encountered outside of corpuscles possessed a single nucleus. The one parasite which contained two nuclei (Pl. X, Fig. 28) may have issued prematurely from the corpuscle owing possibly to mechanical injury to the latter in preparing the blood-film. Of the uninucleate free parasites five occurred singly, eight in couples, and four in one group, suggesting in the latter instances that the parasites had just escaped from corpuscles. From the appearance of the films, many corpuscles were injured when the smear was prepared, consequently no great stress can be laid upon the number of free forms encountered. In the absence of observations upon the living parasite, it is impossible to know in what manner the parasites enter the corpuscles they invade.

Haemogregarina canis adusti n.sp.

Plate XI, Figs. 1—5.

This parasite, of which only eight specimens were discovered in smears from the jackal's spleen, appears to be the first haemogregarine recorded as occurring in an African carnivore. The parasites measured 10 × 4·3 μ to 13 × 6·3 μ in size. Three of the eight parasites were contained in mononuclear leucocytes whose nuclei appeared compressed or distorted from contact with the parasite (Plate XI, Figs. 4, 5). Some of the parasites showed a distinct cytocyst. Otherwise there is nothing special to note. I herewith append a list of the haemogregarines occurring in mammals; all of them have been discovered of recent years. Whereas some of them occur in red blood corpuscles, others occur in mononuclear leucocytes (whence frequently termed *Leucocytozoa*) during the stage of their development when they are found in the peripheral blood:

Haemogregarina occurring in Leucocytes.

Species and Synonymy	Host	Country	Size of parasite
H. canis (James), 1905 =*L. bentleyi* Adie, 1906	*Canis familiaris*	Assam (Bentley, 1904). Perak, Fed. Malay States (Gerrard, 1906), India	8—10 × 4—5 μ (Bentley, 1905) 11—12 × 4·2—5 μ (Gerrard)
H. canis adusti Nuttall, 1910	*Canis adustus* (Jackal)	British East Africa	10—13 × 4·3—6·3 μ
H. felis domesticae (Patton), 1908	*Felis domesticus*	Madras, India	Not given
H. muris Balfour, 1905–6	*Mus decumanus*	Sudan (Balfour). Perth, W. Australia (Cleland, 1906)	9—10·5 × 4·5 μ
=(?) *L. ratti* Adie, 1906	*Mus rattus*	India	11·7 × 5·6 μ
=(?) *H. perniciosa* (Miller), 1908 =*Hepatozoon perniciosum*	*Mus decumanus* (white rat)	United States of N. America	12 × 6 μ
H. musculi (Porter), 1908	*Mus musculus*	London	10·9 × 5·1 μ
H. funambuli (Patton), 1906	*Funambulus palmarum* (palm squirrel)	India	10 × 5 μ
H. leporis (Patton), 1908	*Lepus nigricollis* (hare)	Madras, India	Not given

Haemogregarina occurring in Red Blood Corpuscles.

Species	Host	Country
H. balfouri Laveran, 1905 =*H. jaculi* Balfour, 1906	*Jaculus gordoni* Jerboa	Sudan
H. gerbilli Christophers, 1905	*Gerbillus indicus* Indian field rat	India
H. dasyuri Welsh, Dalyell & Burfitt, 1908	*Dasyurus viverrinus* "native cat"	Sidney, Australia
H. petauri Welsh & Barling, 1908	*Petaurus sciureus* marsupial flying squirrel	Sidney, Australia

Spirochaeta bovis caffris n.sp.

Plate XI, Figs. 6—9.

The examination of four coverglass preparations made from the blood of the buffalo (p. 108) revealed only four spirochaete-like parasites, all of which are illustrated in the accompanying plate. They differ from any spirochaetes I have hitherto seen in being very broad although they taper uniformly at both ends. Stained by Giemsa, they assumed a violet or violet-blue tint. Whereas the parasite depicted in Fig. 6 shows no internal structure, the others contain very fine chromatin particles. In one parasite (Fig. 9) transverse achromatic bands are observable. Although the parasites vary somewhat in size they are remarkably uniform in shape. I do not know what else to call them than spirochaetes. Their form suggests that in life they may possess an eel-like movement, but this appears unlikely from the fact that the four parasites are all curved similarly. They measured 20 to 25 μ by 1 to 1·5 μ.

It is possible that these parasites belong in the same category as those described and figured in a photomicrograph by Bowhill (1905, p. 7, Pl. XI, Fig. 8) and which he regarded as larval nematodes in the blood of a horse at the Cape. Bowhill's parasites measure approximately 15 μ in length, and, judging from his figure, possess darkly stained granular protoplasm. They appear to have been very numerous in the blood of the horse, which was suffering from biliary fever.

The buffalo parasite also recalls forms described by Edm. and Et. Sergent (1903, p. 1163) and roughly figured by them. The Sergent parasites were discovered in the blood of a native of Algiers who suffered from attacks of perspiration every night but appeared otherwise normal. The parasites occurred in the blood with a definite periodicity, *i.e.* only during the daytime (8 a.m. to 6 p.m.). The man's symptoms resembled those of an "accés larvé de paludisme." This parasite must be rare, since the Sergents failed to find it before in the course of hundreds of routine blood examinations made in hospital patients. The parasites were not detected in fresh blood-films. In stained preparations they measured 36 to 45 μ by 1 to 1·5 μ. As a rule they tapered uniformly at both ends. They appear to be flattened and fairly rigid structures, the curvatures being slight. Stained by azur-eosin they mostly assumed a pale mauve tint. In some well-stained specimens the middle third of the parasite appeared blueish and granular, in others this part (the nucleus?) of the parasite appeared pale, distinctly granular and surrounded by a refringent line. Not more than 25 parasites were encountered in a single blood-film.

REFERENCES.

BOWHILL, J. (I. 1905). Equine piroplasmosis or "biliary fever." *Journ. Hygiene*, V. 7—17, Plates I—III.

SERGENT, EDM. and ET. (17, X. 1903). Sur un nouveau protozoaire parasite ectoglobulaire du sang de l'homme. *Compt. Rend. Soc. Biol.*, Paris, LV. 1163—1165, 1 fig.

DESCRIPTION OF PLATES X AND XI.

The drawings were executed in water-colours. They were drawn to scale with the aid of a Winkel Camera-Lucida and Zeiss 2 mm. (oil immersion) objective: using ocular No. 18. The size of the parasites was determined by the use of a Zeiss stage micrometer scale projected upon the paper upon which the drawings were made.

PLATE X.

Piroplasma rossi n.sp.

× 3600.

With the exception of the parasite depicted in Fig. 1, which was found in a liver-smear, all of the parasites figured were encountered in smears from the spleen of the Jackal.

Intracorpuscular parasites.

Figs. 1—3. Uninucleate parasites showing vacuoles and sharply defined or (Fig. 2) diffuse contour with indications of amoeboid movement. Nucleus compact.

Fig. 4. Large ovoid parasite with central nucleus of irregular flowing form with the chromatin diffused.

Figs. 5—6. Large rounded parasites containing two nuclei distant from each other. These parasites are about to divide.

Fig. 7. Two parasites possessing a somewhat pyriform shape.

Fig. 8. Two parasites showing amoeboid movement and diffused chromatin.

Figs. 9—10. Pairs of parasites. Those in Fig. 10 are very large and each shows a large mass of chromatin, the appearance suggesting that the parasites might well be about to divide into two.

Figs. 11—14. Pairs of parasites with diffuse or irregular chromatin. In the parasite to the right in Fig. 14 the chromatin is drawn out in a thread and becoming concentrated in masses at the ends of the thread.

Fig. 15. The parasite to the left appears to have just undergone simple nuclear division, the connecting thread between the flowing chromatin mass having ruptured. In the parasite to the right the nuclei have already become rounded and lie at opposite poles of the parasite. Both parasites show evidence of amoeboid motion.

Figs. 16—17. Pairs of parasites in which the nuclei have subdivided preparatory to the formation of four intracorpuscular parasites. Achromatic lines occur in the blue-staining protoplasm.

Figs. 18—19. Corpuscles containing three parasites, one of which contains two nuclei at opposite ends of the parasite; it represents a dividing form. Each corpuscle contains a pair of parasites which have just undergone division.

Figs. 20—22. Corpuscles containing four parasites, some of which are amoeboid.

Figs. 23—24. Parasites seen in almost dehaemoglobinized corpuscles.

Fig. 25. A parasite presenting a sharply defined bubble-like appearance with the blue-staining protoplasm condensed peripherally and similarly altered (dividing ?) nucleus to one side.

Free Parasites.

Figs. 26—27. Single free parasites of amoeboid form with rounded, compact nuclei and deeply blue-staining protoplasm.

Fig. 28. Single free parasite with two nuclei, the chromatin being diffusely distributed. The parasite was presumably expelled prematurely from the containing corpuscle.

Figs. 29—30. A pair of free parasites and a group of four free parasites which have just escaped from corpuscles. Some of the parasites appear amoeboid.

PLATE XI.

Haemogregarina canis adusti n.sp.

× 3600

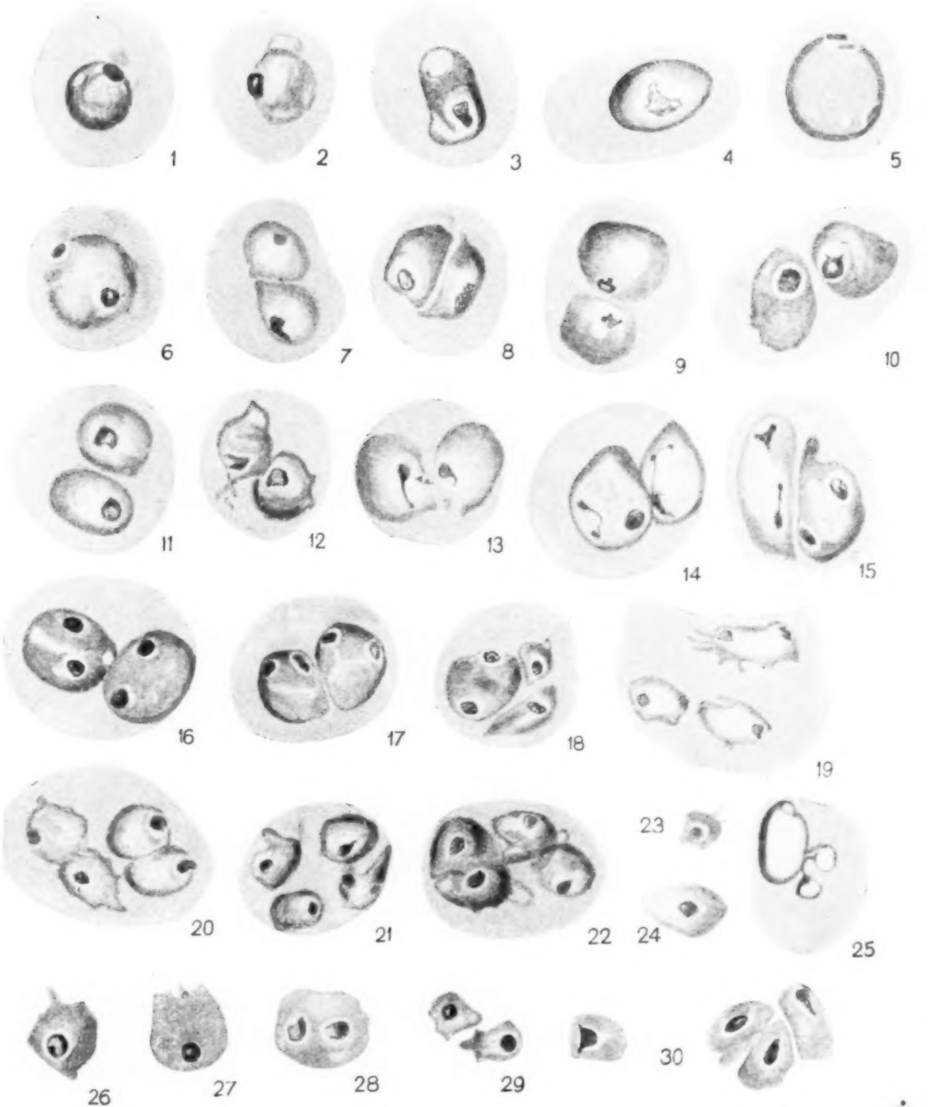
Figs. 1—3. Free parasites. Fig. 3 shows the enveloping membrane or cytocyst and diffuse chromatin; in Fig. 2 there are scattered chromatin points in the protoplasm.

Figs. 4—5. Parasites contained in mononuclear leucocytes whose nuclei are compressed by the body of the parasite. In Fig. 4 the nucleus of the parasite appears reticular.

Spirochaeta bovis caffris n.sp.

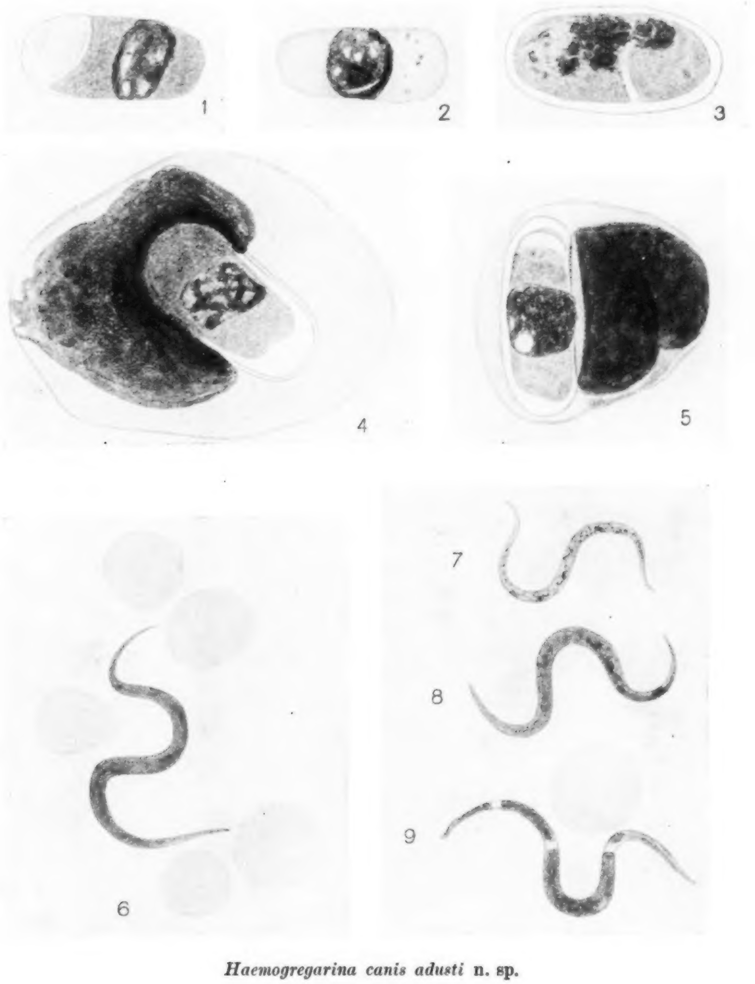
×3600

Figs. 6—9. Spirochaetes showing variation in size but uniformity in shape. Whereas in Fig. 6 the parasite shows no internal structure, in Figs. 7 and 8 the chromatin occurs as fine dust throughout the faintly violet-blue staining protoplasm. In Fig. 9 there are achromatic transverse bands visible.



Piroplasma rossi n. sp.

U of M



Haemogregarina canis adusti n. sp.
and
Spirochaeta bovis caffris n. sp.

U OF M